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PATENT ABSTRACTS OF JAPAN

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(54) ANTIMYCOTIC AGENT FOR EXTERNAL USE

(57)Abstract:
PURPOSE: To obtain an external preparation having excellent skin-penetrability, capable of transferring the drug component to not only corneum but also cuticle and corium and exhibiting excellent remedying effect against profound mycosis as well as latent mycosis by compounding liposome including an antimycotic agent as a main drug component.
CONSTITUTION: An antimycotic agent (e.g. imidazole derivative or antibiotic substance) is dissolved in a solvent (e.g. alcohol or polyhydric alcohol). Ultrasonic vibration is applied to a mixture of the above solution, a phospholipid and water to obtain a liposome containing the antimycotic agent included in the membrane or microsome of the phospholipid. The liposome is compounded as a main drug component. The amount of the antimycotic agent included in the liposome is 0.01-10wt.%, preferably 0.1-5wt.% and the amount of the phospholipid to be used in the formation of liposome is 0.1-10 pts. per. 1 pt. of the antimycotic agent.

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審査請求　未請求　請求項の数 1　(全5頁)

⑭発明の名称　抗真菌外用製剤

⑳特　願　平1-24640
㉒出　願　平1(1989)2月2日

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明細書

1. 発明の名称

抗真菌外用製剤

2. 特許請求の範囲

抗真菌剤を内包したリポソームを主剤成分として配合したことを特徴とする抗真菌外用製剤

3. 発明の詳細な説明

〔産業上の利用分野〕

本発明は、抗真菌外用製剤に関する。さらに詳しくは、抗真菌剤をリポソーム化し主剤成分として含有することにより、安全性が優れ、皮膚局所投与の際、その経皮吸収を高め、皮膚の表皮、真皮に薬物が貯留する抗真菌外用製剤に関するものである。

〔従来の技術〕

抗真菌外用製剤としては、ウンデシレン酸、サリチル酸、ヨウ素、トルナフタート、クロトリマゾール、シッカニンなどを含有する、クリーム剤、液剤などが知られている。

〔発明が解決しようとする問題点〕

抗真菌外用製剤を経皮投与する場合、皮膚角質層のバリヤー機能のため薬物の吸収量が少なく充分な薬効は期待できない。実際には、皮膚糸状菌の寄生部位が皮膚角質層に留まる、表在性白癬のみに有効であり、皮膚真皮以下にまで侵入する深在性白癬には全く無効である。そのため、一度皮膚表面は治癒したかのように思われるが、皮膚のターンオーバーとともに再発し治癒しにくいという問題があり、有効な手段は見つかっていない。

〔問題を解決するための手段〕

この様な事情に鑑み、本発明者らは、鋭意研究を重ねた結果、抗真菌剤をリポソーム化して主剤成分として配合することにより、皮膚透過性が良く、薬物が角質層だけでなく、表皮、真皮にまで達し、表在性真菌症だけでなく、深在性真菌症にも優れた治療効果を発揮することを認め本発明を完成するに至った。

すなわち、本発明は、抗真菌剤を内包したリポソームを主剤成分として配合した抗真菌外用製剤に関するものである。

本発明で使用される抗真菌剤とは、イミダゾール誘導体、抗生物質などが挙げられる。イミダゾール誘導体としてはクロトリマゾール、ミコナゾール、エコナゾール、ケトコナゾールなどがある。イミダゾール誘導体は、真菌の細胞膜に対する直接の阻害と、エルゴステロールの合成阻害による作用を持ち、その抗菌スペクトルは、殆ど全ての真菌とブドウ球菌など一部の細菌にも及び、抗菌活性も強く、広く使われている。また、抗生物質としてはシッカニン、ピロールニトリンが挙げられ、その他にもトルナフタート、トリシクラート、シクロピロクスオラミン、サリチル酸、ヨウ素、エキサラミド、ウンデシレン酸などが挙げられる。

本発明のリポソームは、抗真菌剤を溶媒に溶解したもの、リン脂質及び水の3成分に超音波をかけて得られる。このリポソームはリン脂質の二分子膜の一重層あるいは多重層から成る球状の小胞体で、抗真菌剤がリン脂質の膜中または小胞体内に取り込まれた状態（内包）となる。

抗真菌剤を溶解する溶媒にはアルコールや多価アルコールなどが挙げられる。アルコールとしては、エタノール、プロパノール、イソプロパノールなどであり、多価アルコールとしてはポリエチレングリコール300、ポリエチレングリコール400、ポリエチレングリコール600、グリセリン、1，3－ブチレングリコール、プロピレングリコールなどが挙げられる。その他にもミリスチン酸イソプロピル、クロタミトン、アセトン、メチルエチルケトンなどが挙げられる。

また、リポソーム化にはこれ以外にVortexミキサー法、凍結法、界面活性剤除去法、注入法、フレンチプレス法、逆相蒸発法などがあり、抗真菌剤の性質に合わせて適宜選択して、リポソームを調製して配合すれば良い。さらにリポソームの安定化の目的でコレステロール、グルコース、アミノ酸、高級アルコール、非イオン界面活性剤、イオン性界面活性剤などを添加することができる。リポソーム化に用いられるリン脂質は、大豆リン脂質、卵黄リン脂質、水素添加大豆リン脂質、水素添加卵黄リン脂質、合成リン脂質などであり、

1種または2種以上混合して用いることができる。

本発明においてリポソームに内包される抗真菌剤は、薬理活性を考えて0．01～10重量%の割合になるように添加される。好ましくは、0．1～5重量%の割合になるように添加される。リポソーム化に用いられるリン脂質は抗真菌剤に対して0．1～10倍量の濃度になるように配合する。抗真菌剤の濃度は0．01重量%以下の配合量では、効果は期待できず、10重量%以上の配合量では、リポソーム化が困難である。また、リン脂質は抗真菌剤に対して0．1倍量以下の濃度では、抗真菌剤を全てリポソーム化することはできず、10倍量以上では、リン脂質が多すぎてリポソーム化が困難である。

抗真菌剤のマウスに対するLD$_{50}$は、いずれも1000mg／kg以上であった。

〔実施例〕

次に実施例により本発明を更に説明するが、本発明はこれにより限定されるものではない。処方中の数字は重量%を示す。

実施例－1　クリーム

①スクワラン	9．0
②ステアリルアルコール	0．5
③セチルアルコール	0．5
④ポリオキシエチレン（20）ソルビタンモノステアレート	1．5
⑤ソルビタンモノオレエート	2．3
⑥ミリスチン酸オクチルドデシル	8．5
⑦ワセリン	4．0
⑧精製水	33．8
⑨クロタミトン	5．0
⑩ポリエチレングリコール400	5．0
⑪トルナフタート	3．0
⑫水素添加大豆リン脂質	9．0
⑬精製水	17．9
合計	100．0

成分⑪トルナフタートを、成分⑫⑬に溶解したものを、成分⑧⑨に加え、超音波撹拌してリポソームを調製する。

成分①～⑦を80℃に加熱溶解後、予め80℃

に加熱溶解した成分⑧を加え乳化し、３０℃迄冷却する。これに成分⑨～⑫で調製したリポソームを添加し、撹拌混合するとクリームが得られる。

実施例－２　液剤

①エタノール	5．0
②精製水	53．5
③エタノール	10．0
④クロトリマゾール	1．0
⑤水溶添加大豆リン脂質	7．0
⑥精製水	23．5
合計	100．0

成分⑤に成分④クロトリマゾールを溶解したものを、成分⑥に成分⑤を７０℃加熱溶解した中に加え、超音波撹拌してリポソームを調製する。次いで、成分①②を加えて得られる。

実施例－３　液剤

①エタノール	10．0
②グリセリン	4．0
③1，３－ブチレングリコール	3．0
④精製水	40．9
⑤ポリオキシエチレン（１５）セチルエーテル	1．5
⑥精製水	45．0
⑦水添添加卵黄レシチン	6．0
⑧ポリオキシエチレン（６０）硬化ヒマシ油	0．5
⑨ミコナゾール	2．0
⑩精製水	23．5
合計	100．0

成分⑨ミコナゾール、⑩⑪を成分⑫に溶解し、超音波をかけてリポソームを調製する。

成分①～④を８０℃に加熱溶解後、予め８５℃に加熱溶解した成分⑤を加え乳化し、３０℃迄冷却する。これに成分⑥～⑫で調製したリポソームを添加し、撹拌混合するとクリームが得られる。

［発明の効果］

本発明の効果は、抗真菌剤をリポソーム化し主剤として配合することにより、皮膚の局所治療の際、主剤の経皮吸収を高め、皮膚の表皮、真皮に貯留し、優れた薬効を示す抗真菌外用製剤である。

⑪水添添加卵黄リン脂質	3．5
⑫コレステロール	0．1
⑬シッカニン	0．5
⑭イソプロピルアルコール	14．0
⑮精製水	24．0
合計	100．0

成分⑬⑭をエーテルに溶解させたものをナス型フラスコにいれ、エバポレーターによりエーテルを留去する。これに成分⑮を加え６０℃で撹拌する。次に成分⑪⑫を溶解し、３０℃まで冷却してリポソームを調製した。成分①～④を撹拌溶解後、成分④及び成分⑤～⑫で調製したリポソームを加え、撹拌混合して液剤を得る。

実施例－４　クリーム

①スクワラン	7．0
②ミリステン酸オクチルドデシル	5．0
③サラシミツロウ	3．0
④流動パラフィン	2．0
⑤グリセリン	2．0
⑥ソルビタンモノオレエート	2．5

次に、本発明の効果について動物実験、濾度分布試験、臨床試験及び増強試験の結果を示す。

（動物実験）

実施例－１のクリーム及び下記の比較例－１のクリームについて各２０匹ずつ２日前に毛を刈り取ったモルモット背部１０×１０ｃｍに、皮膚糸状菌であるTrichophyton Rubrumの懸濁液０．５ｍｌを綿棒に塗擦し感染させた。この懸濁液は、感染２４時間前に吸光度０．４を示す真菌懸濁液と、Nervius nutrient brothを２：１の割合で混合させて調製し、２８℃でインキュベートしたものである。治療は感染後第３日目から１日１回７日間、実施例－１及び比較例－１のクリームを１ｇ感染部位に投与し、感染部の状態の変化について目視にて観察した。その結果を表－１に示す。

比較例－１　クリーム

実施例－１のクリームより、成分⑩をリポソーム化せずそのまま配合して実施例－１と同様にクリームを調製した。

表　1の結果より、抗真菌剤トルナフタートをリポソーム化して配合したクリームは、皮膚糸状菌であるTrichophyton Rubrumに対し良好な治癒効果を示し、抗真菌外用製剤として有効なことが分かる。また、実施例－2、3、4においても同様な結果を得た。

表　1　治効実験結果

	実施例－1	比較例－1
匹　数	20	20
著　効	15	10
有　効	4	6
やや有効	1	2
無　効	0	2
有効率	19/20=95%	16/20=80%

（濃度分布試験）

実施例－2及び下記の比較例－2の液剤について^{14}Cで標識したクロトリマゾールを用い、皮膚透過性、濃度分布及び経皮吸収について、毛を刈り取ったラットの背部5×10cm²に、0.5ml1を塗布し、12時間作用させた後の濃度分布をオートラジオグラフィーにて測定した結果を表2に示す。

比較例－2

実施例－2より成分③に、成分④⑤を溶解させリポソーム化せず、成分①②⑥にそのまま配合して実施例－2と同様に調製した。

表　2の結果より、抗真菌剤クロトリマゾールをリポソーム化し配合した液剤は、経皮吸収性に優れ、皮膚の表皮、真皮にまで達し、表在性真菌症だけでなく深在性真菌症にも有効なことが分かる。また、実施例－1、3、4も同様に良好な結果を得た。

以下余白

表　2　組織内濃度分布

部位		実施例－2	比較例－2
		濃度 mcg/cm³	濃度 mcg/cm³
表皮	角質層	70～120	30～80
	有　層	30～60	3～6
真皮	乳頭層	40～60	1～2
	網状層	20～30	0.1～0.5
皮下組織		＜10	＜0.1

（臨床試験）

臨床試験に当たっては、ボランティアを募り、この中で白癬症にかかっており、何れも検鏡で菌陽性の人、48名を対象とし、足白癬に限定した。実施例－3及び下記の比較例－3の液剤について、1日2回適量を患部位に投与し、観察期間は2週間を一応の基準とした。副作用については、接触皮膚炎はもちろんのこと、塗布時の刺激感、発赤、掻痒感などについても記載した。その結果を表　3、4に示す。

比較例－3

実施例－3の液剤より成分⑦をリポソーム化せず、そのまま配合して実施例－3と同様に液剤を調製した。

表　3、4の結果より抗真菌剤シッカニンをリポソーム化し配合することにより、足白癬に対して優れた治癒効果を示し、副作用も少ないことが分かる。また、実施例－1、2、4についても同様に良好な結果を示した。

以下余白

表　3　効果判定

	実施例－3	比較例－3
症　例	25	23
著　効	14	6
有　効	7	8
やや有効	4	6
無　効	0	3
有効率	21/25=84%	14/23=61%

以下余白

表　4　副作用

		実施例－3	比較例－3
症　例		25	23
副作用例数		1	6
副作用の種類	刺激感	0	2
	発　赤	1	2
	湿疹感	0	1
	皮膚炎	0	1

（培養試験）

実施例－4のクリーム及び下記の比較例－4のクリームについて各10羽ずつ1日前に毛を刈り取ったウサギ背部10×20cm²に、カンジダ菌であるCandida albicansを1ml当り1～3×10⁹個含む懸液2mlを塗布し感染させた。治療は感染後第2日目から1日2回10日間、実施例－4及び比較例－4のクリーム2gを感染部位に投与した。その後、皮膚を剥離し表皮組織及び真皮組織の一部を培養し菌の検出を試みた。培養成績は、3日、5日、7日、14日、及び28日目（培養日数）にそれぞれ判定した。

Candida albicansの菌を認めた匹数を表　5に示した。

比較例－4　クリーム

実施例－4のクリームより成分⑪をリポソーム化せず配合して、実施例－4と同様にクリームを調製した。

表　5の結果より、抗真菌剤ミコナゾールをリポソーム化して配合することにより、皮膚中のカンジダ菌の検出も少なく、良好な治療効果を示すことが分かる。また、実施例－1、2、3においても同様な結果を得た。

表　5　培養試験結果

	実施例－4		比較例－4	
	表　皮	真　皮	表　皮	真　皮
3日目	0	0	0	0
5日目	0	0	2	2
7日目	0	1	2	3
14日目	1	1	3	3
28日目	1	2	4	4

Specification

1. Title of the Invention

Anti-fungal preparation for external application

2. Scope of Claim for a Patent

An anti-fungal preparation for external application characterized by comprising as a main component an anti-fungal agent encapsulated in liposomes.

3. Detailed Explanation of the Invention

[Industrial Field of Utilization]

The present invention relates to an anti-fungal preparation for external application. More specifically, the present invention relates to an anti-fungal preparation for external application with a high degree of safety and capable of improving the percutaneous absorption to retain the medication in epidermis and dermis of the skin when topically applied to the skin.

[Prior Art]

Anti-fungal preparations for external application in the form of an ointment, lotion or the like are known which comprise undecylenic acid, salicylic acid, iodine, tolnaftate, clotrimazole, siccanin and the like.

[Problems to be Solved by the Invention]

When the anti-fungal preparation for external application is percutaneously administered, the amount of the absorbed medication is insufficient because of the barrier function of horny layer of the skin, so that sufficient efficacy of the medication cannot be obtained. In fact, such an anti-fungal preparation for external application has activity only against superficial ringworm which is characterized in that a portion of the skin parasitized by dermatophyte is limited to the horny layer of the skin, and exhibits no activity against deep-seated ringworm in which the dermatophyte penetrates into the dermis or underneath the dermis. Therefore, there is the problem that a perfect cure cannot easily be obtained because the symptom is coming back again in conjunction with turnover of the skin cells even though the surface of the skin once appears to be cured. No effective means has been found.

[Means for Solving the Problems]

The inventors of the present invention have made intensive researches in consideration of the above-mentioned circumstances. As a result, it has been found that skin penetration performance is improved by blending as a main component an anti-fungal agent encapsulated in liposomes, so that the medication can stay on the horny layer and further extend to the epidermis and dermis, whereby excellent curative properties against both superficial mycosis and deep-seated mycosis can be exhibited. The present invention has been thus accomplished.

Namely, the present invention relates to an anti-fungal preparation for external application comprising as a main component an anti-fungal agent encapsulated in liposomes.

The anti-fungal agent for use in the present invention includes imidazole derivatives, antibiotics and so on. Examples of the imidazole derivatives are clotrimazole, miconazole, econazole, ketoconazole, and the like. Such imidazole derivatives work to directly block the cell membranes of fungi, and also have an inhibiting effect on ergosterol synthesis. The imidazole derivatives exhibit antibacterial spectra including almost all kinds of fungi and extending to a part of bacteria such as Staphylococcus and the like, and their antimicrobial activities are strong, so that those derivatives are widely employed. In addition, the antibiotics include siccanin and pyrrolnitrin, and in addition, tolnaftate, tolciclate, cyclopiroxolamine, salicylic acid, iodine, exalamide, undecylenic acid, and the like.

The liposomes for use in the present invention can be obtained by applying ultrasonic vibration to a mixture of three components, i.e., an anti-fungal agent dissolved in a solvent, phospholipid, and water. The liposomes are spherical vesicles consisting of one or more bilayer phospholipid membranes, and the liposomes are formed in such a configuration that the anti-fungal agent is trapped (encapsulated) in the phospholipid membranes or within the vesicles.

The solvents used for dissolving the anti-fungal agent therein include alcohols, polyols and the like. Examples of the alcohols are ethanol, propanol, isopropanol and the like. Examples of the polyols are polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 600, glycerin, 1,3-butylene glycol, propylene glycol and the like. In addition to the above, there can be employed isopropyl myristate, crotamiton, acetone, methylethyl ketone and so on.

To obtain the liposomes, there are various methods in addition to the above, for example, the vortex mixing method, thin-film forming method, surfactant removing method, injection method, French press method, reverse phase evaporation method and the like, from which a proper method may be selected depending upon the characteristics of the anti-fungal agent, so as to prepare the liposomes before

blending. Further, cholesterol, glucose, amino acid, higher alcohol, nonionic surfactant, ionic surfactant and the like may be added for the purpose of stabilizing the liposomes. The phospholipid used to obtain the liposomes includes soy-bean phospholipid, egg-yolk phospholipid, hydrogenated soy-bean phospholipid, hydrogenated egg-yolk phospholipid, synthetic phospholipid and the like, and those phospholipids may be used alone or two or more kinds may be used in combination.

In the present invention, the anti-fungal agent to be encapsulated in the liposomes is added in an amount of 0.01 to 10% by weight, preferably 0.1 to 5% by weight, in consideration of the pharmacological activity. The phospholipid used for preparation of the liposomes is added to obtain such a concentration where the amount of the phospholipid may be 0.1 to 10 times that of the anti-fungal agent. If the anti-fungal agent is mixed in an amount of 0.01% by weight or less, desired effects cannot be obtained. When the anti-fungal agent is mixed in an amount of 10% by weight or more, preparation of the liposomes is made difficult. Further, if the amount of the phospholipid is 0.1 times or less that of the anti-fungal agent, the entire anti-fungal agent cannot be encapsulated in the liposomes. On the other hand, when the amount of the phospholipid exceeds 10 times that of the anti-fungal agent, too much phospholipid will make the preparation of liposomes difficult.

Any anti-fungal agents exhibited a LD_{50} in mice of 1000 mg/kg or more.

[Examples]

The present invention will now be explained in more detail with reference to Examples, which are not intended to be limiting the present invention. The numbers given in the formulations indicate percentage by weight.

Example 1 Cream product

1. Squalane	9.0
2. Stearyl alcohol	0.5
3. Cetyl alcohol	0.5
4. Polyoxyethylene (20) sorbitan monostearate	1.5
5. Sorbitan monooleate	2.3
6. Octyldodecyl myristate	8.5
7. Vaseline	4.0
8. Purified water	33.8
9. Crotamiton	5.0
10. Polyethylene glycol 400	5.0
11. tolnaftate	3.0
12. Hydrogenated soy-bean phospholipid	9.0

13. Purified water	17.9
Total	100.0

The component 11 (tolnaftate) dissolved in a mixture of the components 9 and 10 was added to the components 12 and 13 and the obtained mixture was subjected to ultrasonic stirring, whereby liposomes were prepared.

The components 1 through 7 were heated to 80℃ and dissolved, and thereafter the component 8 previously heated to 80℃ and dissolved was added to the mixture of the components 1 through 7 to cause emulsification. Then, the mixture was cooled to 30℃. The liposomes prepared using the components 9 through 13 were added to the above mixture, followed by stirring and mixing, so that a cream product was obtained.

Example 2 Lotion product

1. Ethanol	5.0
2. Purified water	53.5
3. Ethanol	10.0
4. Clotrimazole	1.0
5. Hydrogenated soy-bean phospholipid	7.0
6. Purified water	23.5
Total	100.0

The component 4 (clotrimazole) dissolved in the component 3 was added to a mixture obtained by dissolving the component 5 in the component 6 at 70℃, and the obtained mixture was subjected to ultrasonic stirring to prepare liposomes. Subsequent addition of the components 1 and 2 provided a lotion product.

Example 3 Lotion product

1. Ethanol	10.0
2. Glycerin	4.0
3. 1,3-butylene glycol	3.0
4. Purified water	40.9
5. Hydrogenated egg-yolk phospholipid	3.5
6. Cholesterol	0.1
7. Siccanin	0.5
8. Isopropyl alcohol	14.0
9. Purified water	24.0
Total	100.0

The components 5 and 7 dissolved in ether were placed in an evaporation flask, and the ether component was distilled away using an evaporator. To the resultant mixture, the component 9 was added, followed by stirring at 60℃. Subsequently, the components 6 and 8 were dissolved in the above mixture and the obtained mixture was then cooled to 30℃ to prepare liposomes. After the components 1 through 3 were stirred and dissolved, the component 4 and the liposomes prepared using the components 5 through 9 were added to the mixture of the components 1 through 3, followed by stirring and mixing, so that a lotion product was obtained.

Example 4 Cream product

1. Squalane	7.0
2. Octyldodecyl myristate	5.0
3. Refined beeswax	3.0
4. Liquid paraffin	2.0
5. Glycerin	2.0
6. Sorbitan monooleate	2.5
7. Polyoxyethylene (15) cetyl ether	1.5
8. Purified water	45.0
9. Hydrogenated egg-yolk lecithin	6.0
10. Polyoxyethylene (60) hydrogenated castor oil	0.5
11. Miconazole	2.0
12. Purified water	23.5
Total	100.0

The component 11 (miconazole) and the components 9 and 10 were dissolved in the component 12, and the obtained mixture was subjected to ultrasonic vibration to prepare liposomes.

The components 1 through 7 were heated to 80℃ and dissolved, and thereafter the component 8 previously heated to 80℃ and dissolved was added to the mixture of the components 1 through 7 to cause emulsification. Then, the mixture was cooled to 30℃. The liposomes prepared using the components 9 through 12 were added to the above mixture, followed by stirring and mixing, whereby a cream product was obtained.

[Effects of the Invention]

The effects of the present invention result from the preparation in which the anti-fungal agent encapsulated in liposomes is blended as a main component,

thereby providing an anti-fungal preparation for external application capable of enhancing the percutaneous absorption of the main component and retaining the main component in the epidermis and dermis of the skin, and exhibiting excellent efficacy when topically applied to the skin for treatment.

Then, the effects of the invention will be demonstrated by the results of experiments with animals, concentration distribution tests, clinical trials and incubation tests.

(Experiments with Animals)

Twenty guinea pigs which had been made hairless two days before were separately used for the group of the cream of Example 1 and the group of a cream obtained in Comparative Example 1 shown below. 0.5 ml of a suspension of Trichophyton rubrum, i.e., one of the dermatophytes, was applied to the area of 10 x 10 cm^2 on the back portion of each guinea pig and rubbed in gently to cause fungal infection. The above-mentioned suspension was prepared 24 hours before the infection by mixing a fungi suspension showing an absorbance of 0.4 with Nervina nutrient broth at a ratio of 2:1, followed by incubation at 28℃. Treatment was provided in such a manner that the cream product of Example 1 or Comparative Example 1 in an amount of 1 g was given to the infected area once a day over a period of 7 days from the third day after infection. The change in the condition of the infected area was visually observed. The results are shown in Table 1.

Comparative Example 1 Cream product

A cream was prepared in the same manner as in Example 1 except that the component 11 used in the formulation for the cream product of Example 1 was not encapsulated in the liposomes and mixed as it was.

As can be seen from the results of Table 1, the cream product comprising the anti-fungal agent, i.e., tolnaftate encapsulated in liposomes exhibits an excellent curing effectiveness against the dermatophyte, Trichophyton rubrum, and is therefore found to be effective as an anti-fungal preparation for external application. Further, Examples 2, 3 and 4 produced similar results.

Table 1 Results of Animal Experiments

	Example 1	Comparative Example 1
Number of guinea pigs	20	20
Significantly effective	15	10
Effective	4	6
Slightly effective	1	2
Ineffective	0	2
Effectiveness	19/20=95%	16/20=80%

(Concentration Distribution Tests)

Using ^{14}C-labelled clotrimazole, the lotion of Example 2 and a lotion obtained in Comparative Example 2 shown below were investigated for the skin permeability, concentration distribution and percutaneous absorption in such a manner that 0.5 ml of the lotion was applied to the area of 5 x 10 cm^2 on the back portion of each hairless rat and then the concentration distribution was determined by means of autoradiography after the agent was allowed to work for 12 hours. The results are shown in Table 2.

Comparative Example 2

A product was prepared in the same manner as in Example 2 except that the components 4 and 5 were not dissolved in the component 3 to prepare the liposomes, but mixed with the components 1, 2 and 6 as they were.

As can be seen from the results of Table 2, the lotion comprising the anti-fungal agent, i.e., clotrimazole encapsulated in liposomes exhibits excellent percutaneous absorption, so that the agent can extend to the epidermis and the dermis of the skin. Therefore, the above-mentioned lotion is found to have effectiveness against not only superficial dermatophytosis, but also deep-seated dermatophytosis. Further, Examples 1, 3 and 4 similarly produced good results.

Table 2 Concentration Distribution within Tissues

		Example 2	Comparative Example 2
		Concentration (mcg/cm^3)	Concentration (mcg/cm^3)
Epidermis	Horny layer	70 - 120	30 - 80
	Prickle-cell layer	30 - 60	3 - 6
Dermis	Papillary layer	40 - 60	1 - 2
	Reticular layer	20 - 30	0.1 - 0.5
Subcutaneous tissue		< 10	< 0.1

(Clinical Trials)

Volunteers were recruited for the clinical trials. Among the volunteers, 48 subjects were selected who were suffering from ringworm, particularly limited to interdigital ringworm, and proved positive for the fungi on microscopic examination. An appropriate dose of the lotion of Example 3 or a lotion obtained in Comparative Example 3 shown below was applied to the infected area twice a day. The observation time was basically set to two weeks. The side effects were recorded with respect to a burning sensation at the application, rubefaction and itch, as well as contact dermatitis. The results are shown in Tables 3 and 4.

Comparative Example 3

A lotion product was prepared in the same manner as in Example 3 except that the component 7 used in the formulation for the lotion of Example 3 was not encapsulated in the liposomes and mixed as it was.

As can be seen from the results of Tables 3 and 4, when the anti-fungal agent, i.e., siccanin in the form of liposomes is blended, excellent curing effectiveness against Trichophyton is exhibited and the side effects are found to be reduced. Further, Examples 1, 2 and 4 similarly produced good results.

Table 3 Assessment as to Efficacy

	Example 3	Comparative Example 3
Number of cases	25	23
Significantly effective	14	6
Effective	7	8
Slightly effective	4	6
Ineffective	0	3
Effectiveness	21/25=84%	14/23=61%

Table 4 Side Effects

		Example 3	Comparative Example 3
Number of cases		25	23
Number of cases where side effects were caused		1	6
Types of side effects	Burning sensation	0	2
	Rubefaction	1	2
	Itch	0	1
	Dermatitis	0	1

(Incubation Tests)

Ten rabbits which had been made hairless the day before were separately used for the group of the cream of Example 4 and the group of a cream obtained in Comparative Example 4 shown below. 2 ml of a solution containing Candida albicans at a concentration of 1×10^3 to 3×10^3 per milliliter was applied to the area of 10×20 cm^2 on the back portion of each rabbit to cause fungal infection. Treatment was provided in such a manner that the cream of Example 4 or Comparative Example 4 in an amount of 2 g was applied to the infected area twice a day over a period of 10 days from the second day after infection. Thereafter, the skin was peeled off and a part of the epidermis tissue and a part of the dermis tissue were subjected to incubation to check the presence of the fungi. The incubation results were assessed on each of the 3rd, 5th, 7th, 14th and 28th days (the number of days for incubation).

The number of rabbits where the fungi of Candida albicans were recognized is given in Table 5.

Comparative Example 4 Cream product

A cream product was prepared in the same manner as in Example 4 except that the component 11 used in the formulation for the cream of Example 4 was not encapsulated in liposomes, but mixed as it was.

As can be seen from the results of Table 5, when the anti-fungal agent, i.e., miconazole in the form of liposomes is blended, the number of fungi of Candida albicans recognized in the skin is reduced, which demonstrates an excellent curing effect. Further, Examples 1, 2 and 3 produced similar results.

Table 5 Results of Incubation Tests

	Example 4		Comparative Example 4	
	Epidermis	Dermis	Epidermis	Dermis
3rd day	0	0	0	0
5th day	0	0	2	2
7th day	0	1	2	3
14th day	1	1	3	3
28th day	1	2	4	4

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